

HTM Explorer

R-based software for inspection and analysis of image-derived data

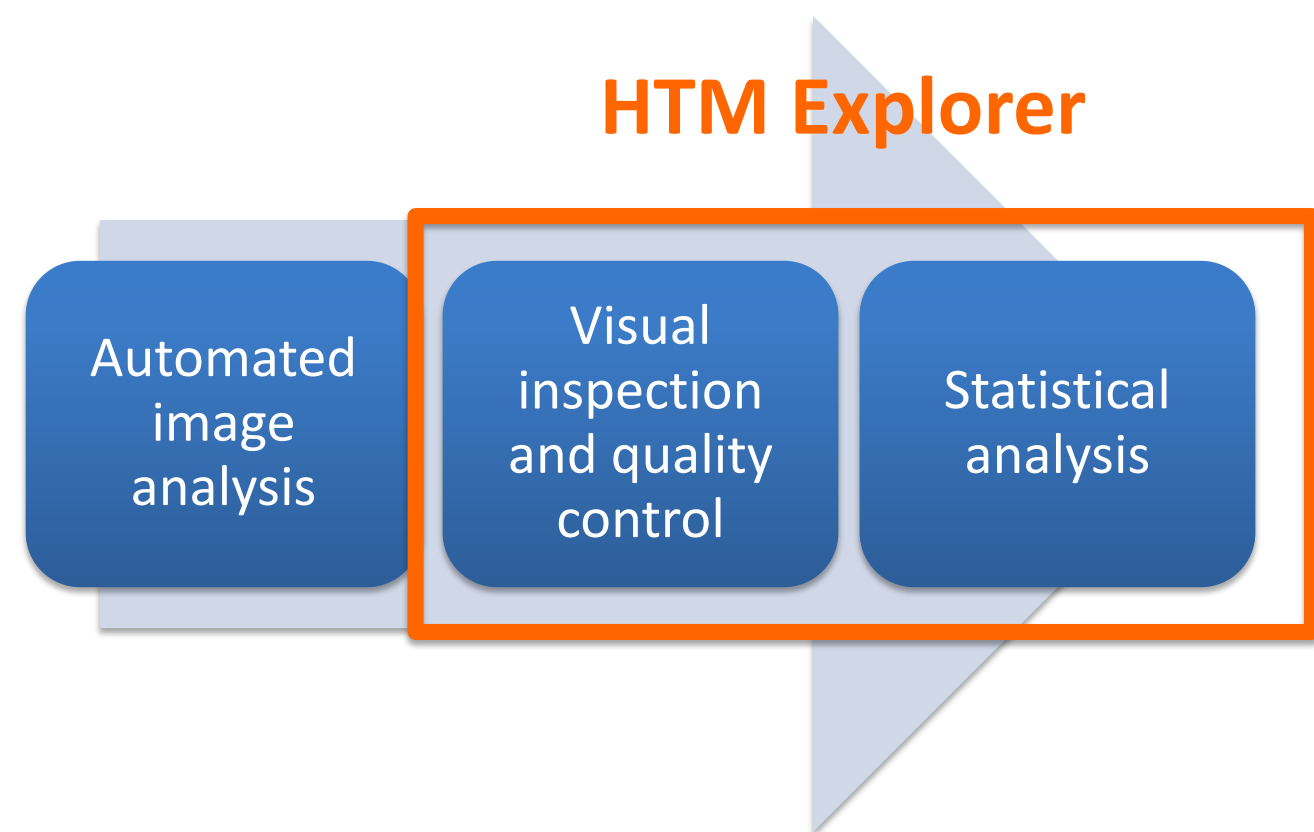


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Motivation



Automated image analysis typically results in big results tables that require quality control and statistical analysis.

Typical steps are:

- Identify images where image analysis failed (=> improve image analysis)
- Check data for spatial effects, e.g., cell seeding... (=> improve experimental conditions)
- Perform image-based quality control (e.g. focus quality and cell density)
- Compute statistical significance of treatment effects

Our R-based HTM Explorer software facilitates this workflow by:

- Ease of use to perform standard tasks via a GUI
- Plotting: interactive (Zoom and Click&View) and annotated (QC, treatment, ...)
- Click&View functionality displays the images associated with each data point in ImageJ
- Freedom to perform non-standard analysis via the R-console

Shortcomings of some otherwise very good existing solutions (as of 2 years ago):

cellHTS2: R-package for console-based HTM data analysis

- Requires coding (no GUI)
- No plotting with link back to the images
- Finished project => small adaptations hardly possible (e.g., current version required same number of replicates for each layout ...)

CellProfiler Analyst: Python-based GUI for HTM data analysis

- No access to data without GUI (=> not flexible)
- No statistical analysis
- Limited image raw data interaction

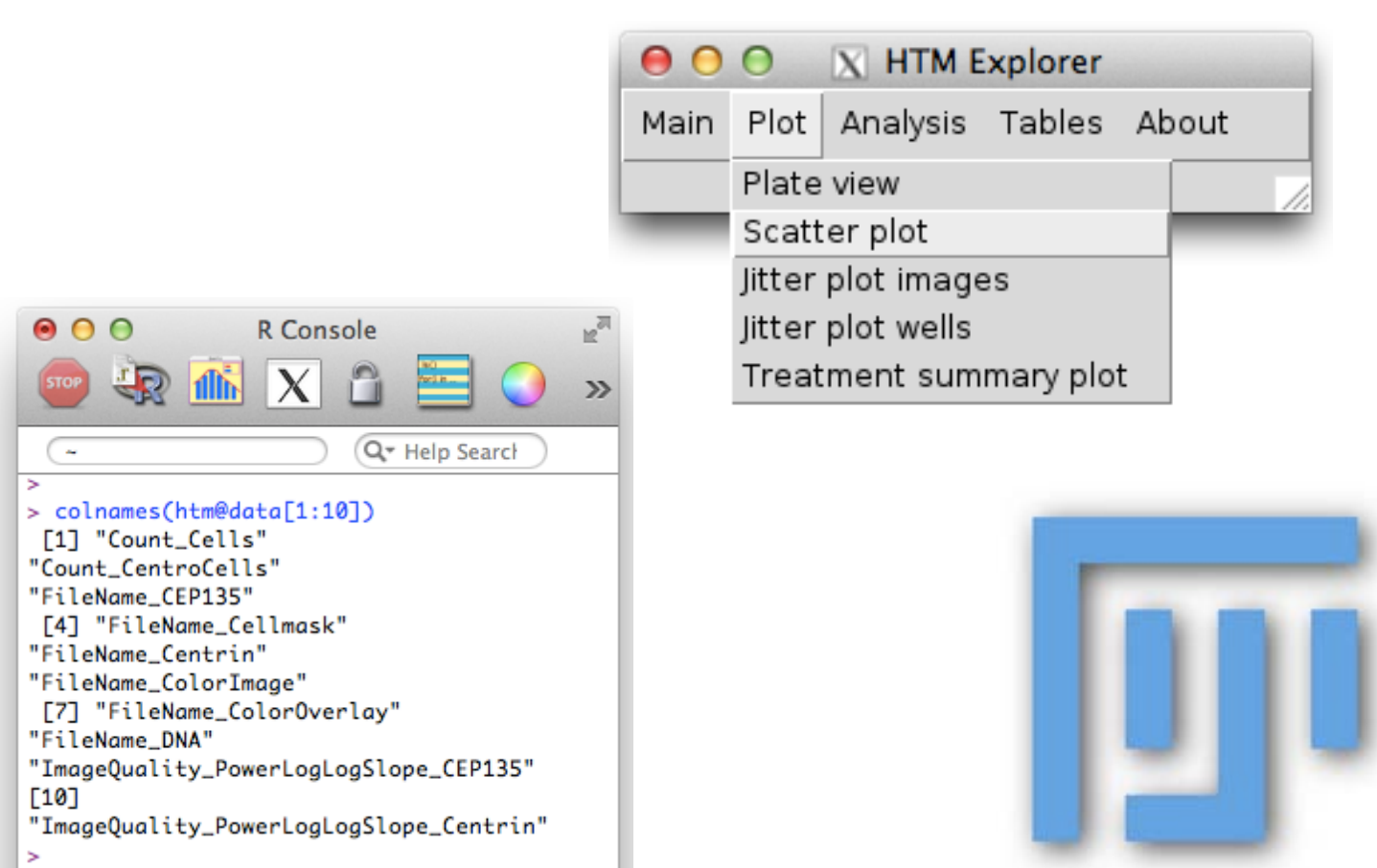
SpotFire:

- Commercial => difficult to distribute
- Limited image raw data interaction
- Statistical tools?
- Scripting access to data?

Design: R, R-GUI, and ImageJ

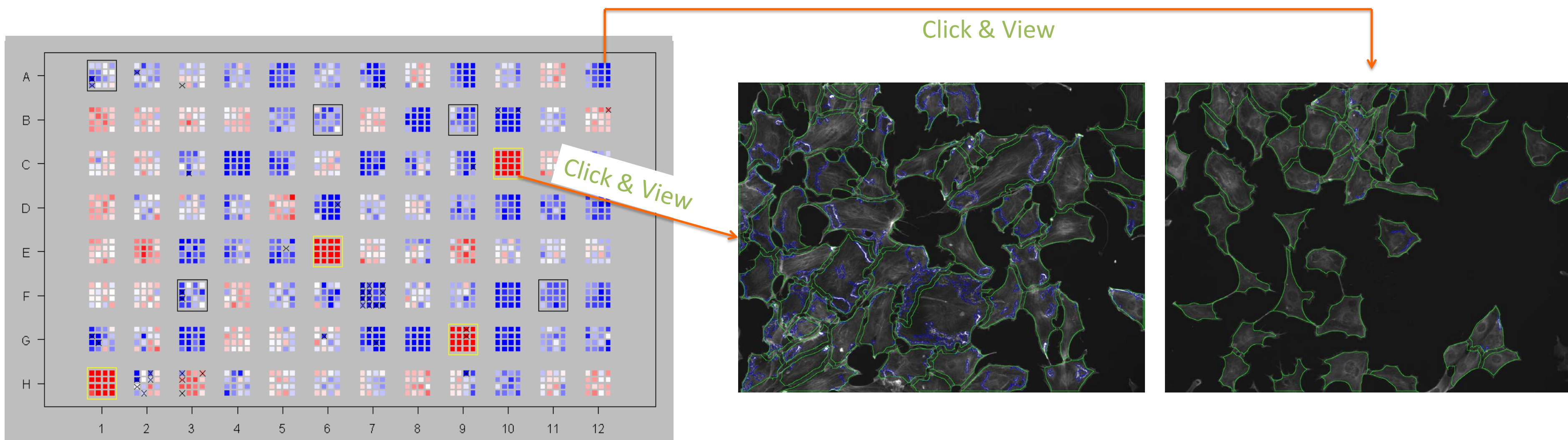
Access to data and HTM Explorer functions from both GUI and R console
=> convenient use and flexibility

Image display with ImageJ:
=> access to pixel values
=> measurement tools
=> support for all image formats (bioformats)



Interactive and Annotated Plotting

Visualize spatial effects and check efficiency of control treatments



Data Loading and Configuration

Input: one table where each row contains data for one image (incl. paths to raw data)

	A	B	C	D	E	F	G
1	Metadata_treatment	Count_Cells	Filename_DNA	Filename_RFP	Filename_Tiemo	ImageQuality_PowerLogSlope_DNA	
2	s229174-Scramble	5	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.205478056	
3	empty-empty	63	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.091430255	
4	s229174-Scramble	30	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.014917843	
5	empty-empty	43	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.186473448	
6	s229174-Scramble	41	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.086124049	
7	empty-empty	49	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.084427563	
8	s229174-Scramble	46	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.089749401	
9	empty-empty	50	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			1.898484869	
10	s229174-Scramble	13	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.044484542	
11	empty-empty	83	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			1.948564633	
12	s229174-Scramble	37	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.249394949	
13	empty-empty	69	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.009751184	
14	s229174-Scramble	33	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.249394949	
15	empty-empty	39	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.132309051	
16	s229174-Scramble	39	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.132309051	
17	empty-empty	39	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.132309051	
18	s229174-Scramble	39	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.132309051	
19	empty-empty	39	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.132309051	
20	s229174-Scramble	39	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.132309051	
21	empty-empty	75	Pilot_screen_autophagy_01-empty-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.044484542	
22	s229174-Scramble	75	Pilot_screen_autophagy_01-s229174-Pilot_screen_autophagy_01-Pilot_screen_autophagy_01			2.044484542	

Select column names containing ...

Treatment: Metadata_siRNA_gene

Experiment (---Plate): Metadata_PlateName

Well coordinate: Metadata_WellNum

Position coordinate: Metadata_PosNum

Close

Monitor out-of-focus images

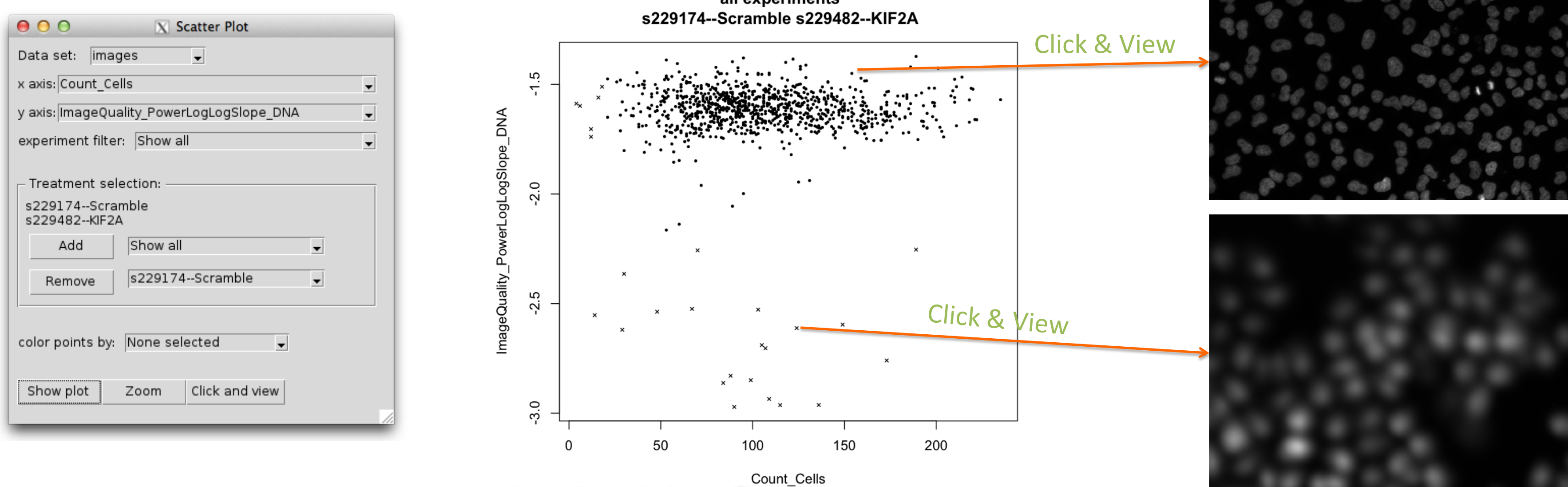


Image Filtering

Add Image QC

Measurement:

ImageQuality_PowerLogSlope_DNA

Minimum: -2.2

Maximum: 1000000

Add QC

Remove Image QC

Count_Cells min=20 max=1e+06

Remove QC

Apply Image QCs Now

Statistical Analysis

Median_z_score:

- Mean and SD based z-score against controls of same batch
- Median z-score of all batches

Median_robust_z_score:

- Median and MAD based z-score against controls of same batch
- Median z-score of all batches

ANOVA:

- For each treatment: t-test against all controls („reference“) occurring in same batches as the treatment; with batch-effects as „blocking factor“
- Implementation using R-commands: **lm()** and **summary()**

	A	B	C	D	E	F	G	H	I	J	K	L
1	controls		ANOVA_pValue	ANOVA_signCode	ANOVA_estmate	ANOVA_std_error	median_z_score	median_robust_z_score	mean_objects_per_image	numObjectsOK	numImagesOK	numReplicatesOK
2	s229174-Scramble	46483-S4556	4.16E-07 ***		-0.003078976	0.000262046	-2.292740451	-2.286999384	138.1452632	65619	475	30
3	s229174-Scramble	42039-C3142	2.19E-09 ***		-0.00621841	0.00079323	-4.797183536	-4.591830244	138.1452632	2992	16	4
4	s229174-Scramble	42504-ANKR026	6.40E-07 ***		0.000857659	0.001155702	5.117062637	4.247161333	120.65	2413	20	5
5	s229174-Scramble	42863-STL	8.54E-07 ***		-0.004974332	0.000851227	-3.344979469	-4.149738415	147.4736842	2802	19	5
6	s229174-Scramble	42863-STL	2.94E-06 ***		-0.00338992	0.000976335	-3.19679121	-2.93812635	154.25	3085	20	5
7	s229174-Scramble	42915-C2C03	4.53E-06 ***		-0.00445746	0.000804182	-3.332483094	-3.564334923	149.5333333	2243	15	4
8	s229174-Scramble	42974-40A4731	7.36E-06 ***		0.005125129	0.000946586	3.408115981	2.933474733	134.4125	2157	16	4
9	s229174-Scramble	42915-C2C03	1.23E-05 ***		-0.004732914	0.000945691	-2.403570543	-2.287194921	166.8	3336	20	5
10	s229174-Scramble	42015-DYNCH14	1.26E-05 ***		0.004365705	0.000836822	3.057487362	2.725209052	111.5625	1785	16	4
11	s229174-Scramble	42946-S0A4	2.41E-05 ***		-0.004074873	0.000816277	-2.634466163	-3.183354594	177.375	2838	16	4
12	s229174-Scramble	41878-COP110	2.87E-05 ***		0.003934146	0.000803047	3.226313733	3.636366282	144.8125	2317	16	4
13	s229174-Scramble	4195-AUWA	3.17E-05 ***		-0.00395744	0.000808469	-2.861240421	-3.20170138	154.1875	2467	16	4
14	s229174-Scramble	42515-TTL3	3.72E-05 ***		0.004138349	0.00080705	2.999668859	2.68389731	91.4375	1463	16	4
15	s229174-Scramble	40993-KIF48	4.85E-05 ***		-0.003504978	0.000794957	-2.32345578	-2.57165468	154.5	2472	16	4
16	s229174-Scramble	40074-GIMAP5	5.62E-05 ***		0.004043445	0.00084677	2.205939356	2.08052473	131.25	2100	16	4
17	s229174-Scramble	47839-KIF5A	6.99E-05 ***		0.00452106	0.001016119	2.810184611	3.008792761	111.7894737	2124	19	5
18	s229174-Scramble	423149-C2C02	7.73E-05 ***		-0.003397115	0.000731134	-2.520375339	-3.899771894	139.2666667	2089	15	4
19	s229174-Scramble	40120-KIF24	0.000158439 ***		0.003687355	0.00083779	1.981427134	1.765136917	140.6666667	2107	15	4
20	s229174-Scramble	41038-PLK4	0.000183284 ***		-0.003416061	0.000803883	-2.454991719	-2.453428469	147.4375	2359	16	4
21	s229174-Scramble	420571-DC7N2	0.000323645 ***		-0.003074688	0.00081315	-2.02451281	-2.430071617	159.7894737	3036	19	5
22	s229174-Scramble	1984-BICD1	0.000519219 ***		0.004284421	0.001129566	1.981468819	2.091771811	96.73684211	1838	19	5

Statistical Analysis

Measurement to be analysed: Mean_CentroCells_Math_CentrinNormTot

Method to average images within one well: weighted_mean_of_images

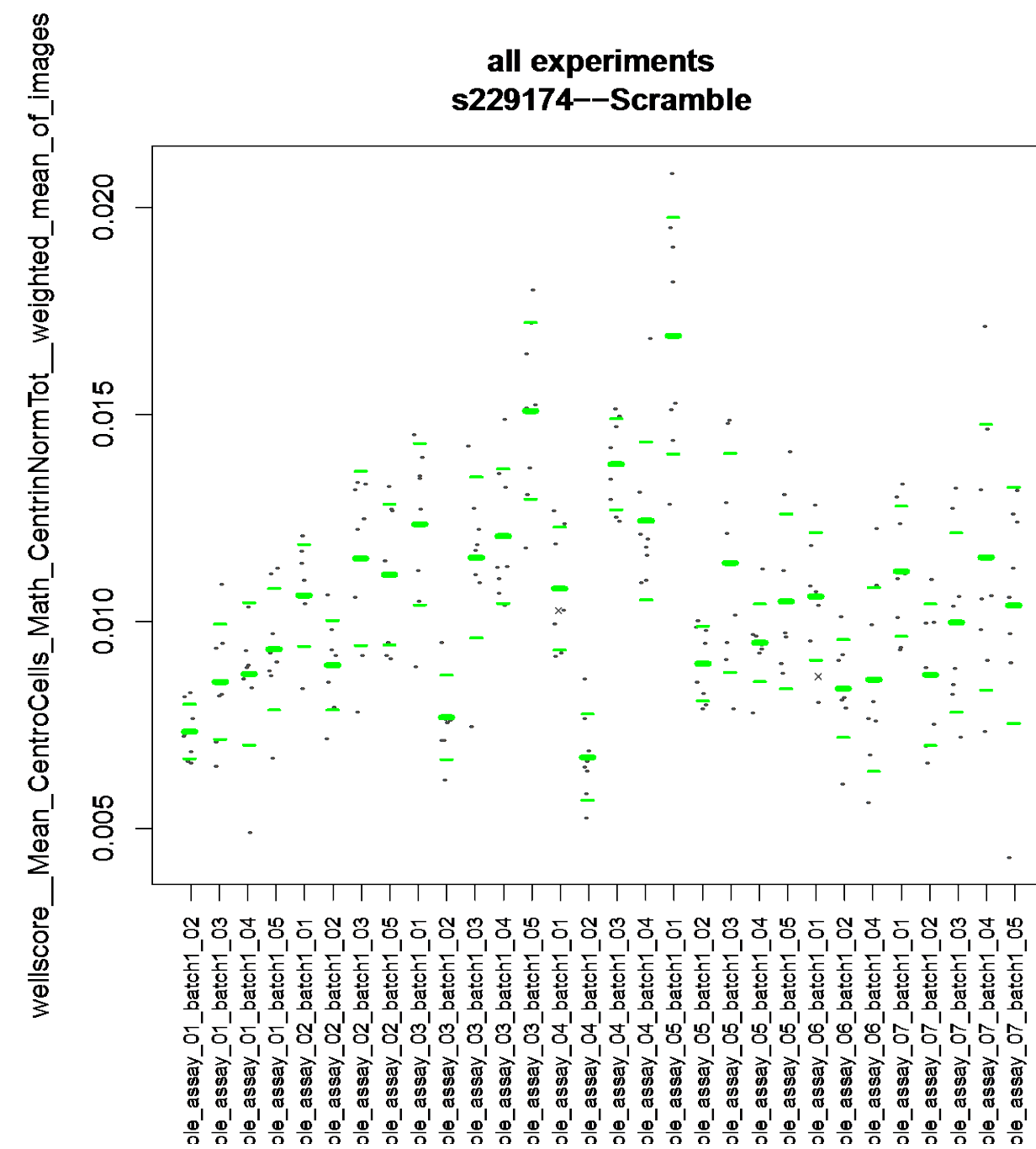
Number of objects per image: Count_Cells

Well QC: Minimum number of valid objects: 100

Negative control: s229174-Scramble

Data transformation: none

Analyze Help Options Close



Usage Experiences

- HTM Explorer has been used in four microscopy-based screening projects, consisting each of 10k-100k images.
- HTM Explorer is readily used by researchers without any programming experience.
- HTM Explorer is independently used by visitors even after they left EMBL.
- The combination of the R-GUI, R-console interface and image display in ImageJ so far has met all requirements.

Acknowledgements

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